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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Substituted Phenylacetonitriles for Breaking Down Resistance

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Substituted phenylacetonitriles for breaking down resistance

The present invention relates to the use of substituted phenylacetonitriles for breaking down resistance to antimalarials.

The development of resistance to chemotherapy, especially multiple resistance, is a serious and continuing medical problem. It may lead to proven drugs becoming completely useless, and often there is no satisfactory alternative available. The way resistance develops is still largely unknown.

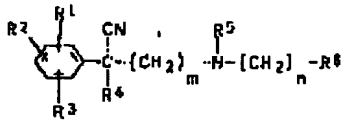
Medicaments which have penetrated into resistant cells may be "pumped" out again or else chemically detoxified by the cells (Spektrum der Wissenschaft, October 1988, pages 30/31).

The observation that verapamil is able to break down resistance in cancer cells (JP-A 83,624/1983) and Plasmodia (Proc. Nat. Acad. Sci. USA, 83 (1987) 7310, Science, 235 (1987) 899) has therefore rapidly led to clinical applications in oncology. In this connection, the known cardiovascular activity of verapamil proved to be a side effect impeding use in practice. The high doses which are required mean that use is possible only under special clinical conditions (intensive care unit) or prevented entirely.

It has furthermore been disclosed that the strong calcium antagonists verapamil and nifedipine also reduce the chemotherapeutic resistance of the agents causing malaria (WO 88/03802).

We have now found that certain substituted phenylacetonitriles can be used advantageously for breaking down resistance.

The present invention relates to the use of those racemic and optically active substituted phenylacetonitriles of the formula I



I,

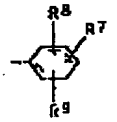
where

$R^1$, $R^2$ and $R^3$ are hydrogen, halogen, $C_{1-4}$-alkyl, $C_{1-4}$-alkoxy, trifluoromethyl, or two adjacent substituents $R^1$ and $R^2$ or $R^2$ are together $-CH_2-CH_2-CH_2-CH_2-$ or $-CH=CH-CH=CH-$,

$R^4$ is saturated or unsaturated alkyl or cycloalkyl of up to 15 carbon atoms or phenyl,

$R^5$ is hydrogen or $C_{1-4}$-alkyl, m and n are each 2, 3 or 4, and

$R^6$ is saturated or unsaturated alkyl of up to 20 carbon atoms, $C_{4-8}$-cycloalkyl or



where $R^7$, $R^8$ and $R^9$ are identical or different and are hydrogen, halogen, $C_{1-4}$-alkyl, $C_{1-4}$-alkoxy, trifluoromethyl, or two adjacent substituents $R^7$ and $R^8$ or $R^8$ are together $-CH_2-CH_2-CH_2-CH_2-$ or $-CH=CH-CH=CH-$, and the salts thereof with physiologically tolerated acids, which

a) have an $EC_{50}$ above $10^{-7}$ M in the calcium antagonism test described in Advances in Myocardiology Vol. 4 (1983) on page 505,

b) have an $ED_{20}$ above 1.0 mg/kg in the test for lowering of blood pressure and

c) have a threshold dose above 2.0 mg/kg in the test for AV blockade,

for the preparation of drugs for breaking down resistance to antimalarials.

"Above $10^{-7}$" means greater than $10^{-7}$, for example

$10^{-6}$.

Resistance to the following antimalarials is of particular concern: chloroquine, hydrochloroquine, quinine, pyrimethamine, mefloquine and primaquine, and combinations thereof.

The following substances are particularly suitable for breaking down resistance to antimalarials:

a. 1-(3-Methoxyphenyl)-3-aza-7-cyano-7-(3,4,5-trimethoxyphenyl)-8-methylnonane hydrochloride

b. (-)-(S)-1,7-diphenyl-3-methylaza-7-cyano-8-methylnonane hydrochloride

c. (+)-1-(3,4-Dimethoxyphenyl)-3-methylaza-7-cyan-7-(3,4,5-trimethoxyphenyl)-8-methylnonane hydrochloride

d. (R)-1-cyclohexyl-3-methylaza-7-cyano-7-(3,4,5-trimethoxyphenyl)-8-methylnonane hydrochloride

e. 1-Cyclohexyl-3-methylaza-7-cyano-7-phenyl-8-methylnonane hydrochloride

f. 7-Methylaza-11-cyano-11-(3,4,5-trimethoxyphenyl)-12-methyltridecan hydrochloride

g. 1,7-Diphenyl-3-aza-7-cyano-8-methylnonane hydrochloride

h. (R)-1-(3-methoxyphenyl)-3-methylaza-7-cyano-7-(3,4-dimethoxyphenyl)-8-methylnonane hydrochloride 2-isopropyl-valeronitrile

i. 1-Cyclohexyl-3-methylaza-7-cyano-7,7-bis(3,4-dimethoxyphenyl)heptane amidosulfonate

j. (+)-(R)-1,7-diphenyl-3-methylaza-7-cyano-8-methylnonane hydrochloride

k. R-Verapamil

Preferred substances are b, c, d, e and k.

The abovementioned substances can, if desired, be in the form of salts thereof with physiologically tolerated acids, of which the following are suitable and preferred: hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, citric acid, malonic acid, salicylic acid, maleic acid, fumaric acid, succinic acid, ascorbic

acid, malic acid, methanesulfonic acid, lactic acid, gluconic acid, glucuronic acid, amidosulfonic acid, benzoic acid and tartaric acid.

The compounds of the formula I can be administered together with or separate from the antimalarials. The compounds I are usually administered orally, whereas the antimalarials (= active substances) are given orally or parenterally (eg. i.v. or i.p.).

The ratio of compound I to active substance depends on the disease to be treated, the condition of the patient and the active substance used. The ratio is normally about 1:1 to 500:1, preferably 0.1:1 to 10:1. The compounds I are usually administered in an amount of from 20 to 2000 mg per patient and day orally and from 10 to 300 mg intravenously or 20 to 500 mg intraperitoneally, per patient and day. The active substances are administered in the amounts stated by the manufacturers to be appropriate for administration of these substances alone.

The substances can be in the form of coated or uncoated tablets or capsules for oral administration or as solutions for injection (i.v., i.p. or i.m.). Solutions can also be infused. The dosage forms are produced by conventional methods.

The calcium antagonism test described in Advances in Myocardiology, Vol. 4 (1983) on page 506 is carried out as follows:

Two test contractions were induced with calcium in $K^+$-depolarised spiral strips of aorta (20 mm long, 2 mm wide) from Sprague-Dawley rats (weighing 250-300 g, anesthetized with ether). The preload was 1 g; relaxation was brought about within about 1 h in Tyrode solution at 37°C, through which was bubbled a mixture of 95% $O_2$ and 5% $CO_2$ (pH 7.3-7.4). The strips were rinsed in Ca-free Tyrode solution containing 0.2 mM NaEDTA for 3 x 5 min. The Ca-free strips were depolarised with Tyrode solution in which 100 mM $Na^+$ had been replaced by $K^+$. After 10 min,

a first contraction was induced with 0.5 mM $Ca^{2+}$. 15 min later, the strips were washed for 10 min with Ca-free Tyrode solution containing 0.2 mM NaEDTA and again depolarized. The drug was administered 15 min before the second test contraction induced with calcium (0.05 mM). The tension was recorded isometrically. The percentage inhibitions of calcium-induced contraction in the presence of various drug concentrations were calculated. Linear regressions were calculated from the linear part of the concentration/effect plot. The relative activity was estimated from the distance between these regression lines.

The lowering of blood pressure was determined after i.v. administration to Sprague-Dawley rats under urethane anesthesia by measuring the mean blood pressure in the carotid artery. The ED 20% was calculated from the dose-effect relations as the dose (mg/kg) which lowered the blood pressure by 20%. Also determined was the threshold dose for blockade of atrioventricular conduction (second degree AV block) in the ECG.

The use of the substituted phenylacetonitriles is advantageous for controlling malaria because they are better tolerated by the cardiovascular system.

Preparation examples

1. 500 mg of (-)-S-1,7-diphenyl-3-methyl-aza-7-cyano-8-methylnonane hydrochloride and 200 mg of chloroquine were dissolved in 250 ml of physiological saline, sterilized and dispensed into an infusion bottle under sterile conditions.

2. Tablets containing the following were prepared:

| | |
|---|---|
| (+)-1-(3,4-dimethoxyphenyl)-3-methyl-aza-7-cyan-7-(3,4,5-trimethoxyphenyl)-8-methylnonane hydrochloride | 100 mg |
| Chloroquine sulfate | 50 mg |
| Auxiliaries | 200 mg |

The efficacy of the combination therapy for

malaria was determined in the following tests.

Test I

Each of the substances was tested initially alone and then combined with the antimalarial on human erythrocytes infected with resistant Plasmodia (Pl. falciparum). The incorporation of radiolabeled hypoxanthine was used as a measure of parasite growth. Using the microdilution technique (Antimicrob. Agents Chemotherapy 16 (1979) 710) the inhibition of hypoxanthine incorporation by the substances according to the invention was found to be zero or only slight. In contrast, a distinctly synergistic inhibition was found in combination with an antimalarial. The FIC index (a mathematical representation of an isobologram) was used for evaluation. A figure of 1.0 means that the effects are additive, and <0.5 indicates synergism. The figure found for racemic verapamil and chloroquine was 0.45.

$$\text{FIC index} = \frac{[A]}{IC_{50A}} + \frac{[B]}{IC_{50B}} \quad (ng/ml)$$

$IC_{50}$ = concentration of test substance producing 50% inhibition of the parasites.

[A] = measured $IC_{50}$ of substance A in the presence of a defined (eg. 500 to 125,000 ng/ml) amount of substance B.

[B] = measured $IC_{50}$ of substance B in the presence of a defined (eg. 500 to 125,000 ng/ml) amount of substance A.

$IC_{50A}$ = $IC_{50}$ of substance A alone

$IC_{50B}$ = $IC_{50}$ of substance B alone.

The results for combinations with chloroquine were as follows:

| Test substance | MIC index |
|---|---|
| R-verapamil | 0.56 |
| b) | 0.19 |
| c) | 0.53 |
| d) | 0.52 |
| g) | 0.19 |
| h) | 0.29 |
| k) | 0.37 |

It was thus shown that addition of the substances according to the invention made chloroquine and other antimalarials once again highly effective against the agents causing malaria, even when resistant.

Test II

To confirm and further differentiate the breaking down of resistance, in the method described in Ann. Trop. Med. Parasit. 72 (1978) 23 (cf. also Exp. Parasitol. 17 (1965) 89 and Ann. Trop. Med. Parasit. 69 (1975) 155), mice were inoculated with parasitised erythrocytes and treated on four consecutive days starting 3 day after the injection. The parasitmia was determined one day after the end of treatment.

1. General test conditions

Male Swiss albino mice free of Eperythrozoon coccoides and weighing from 18 to 20 g were used for all the tests.

They were housed in plastic cages with 5 mice per cage in controlled-temperature (22 ± 2°C) rooms.

Test parasite

P. yoelii NS, obtained from P. yoelii N: moderately resistant to chloroquine. Obtained by cyclic passage through Anopheles stephensi and exposure to the drug in the mouse (60 mg/kg s.c. once during the passage) (for details, see Ann. Trop. Med. Parasit. 72 (1978) 23).

2. Test of the schizontocidal action in blood

Male mice were inoculated intravenously with erythrocytes ($10^7$) parasitized by the NS strain of P. yoelii. The animals were then treated once a day for four consecutive days starting on the day of infection. The compounds were dissolved or suspended in sterile distilled water containing ®Tween 80 and administered subcutaneously. Where preparation of an aqueous solution was very difficult, the test substance was first dissolved in dimethyl sulfoxide and then aqueous dilutions were prepared for use. The parasitmia was determined on the day after the last treatment. The decrease in parasites compared with the untreated controls was calculated by probit analysis of the log dose/effect plot.

Examples

EXAMPLE 1

Mice infected with chloroquine-resistant Plasmodium yoelii ssp. were treated subcutaneously with increasing doses of chloroquine for 4 days as described in II.2.

One day after the last treatment the parasitmia was determined and compared with the controls. Distinct parasitmia persisted even with the high doses of 30 and 60 mg of chloroquine per kg.

TABLE 1

| Chloroquine daily dose mg/kg | Number of mice | Mean control parasitemia | % Parasitemia |
|---|---|---|---|
| 0.3 | 5 | - | 100 |
| 1.0 | 5 | - | 79 |
| 3.0 | 5 | - | 32 |
| 10.0 | 5 | - | 14 |
| 30.0 | 5 | - | 7 |
| 60.0 | 5 | - | 7 |
| 0 | 10 | 23 | 100 |

EXAMPLE 2

In the same test, R-verapamil (in place of chloroquine) had virtually no effect on the parasitemia

TABLE 2

| R-verapamil daily dose mg/kg | Number of mice | Mean control parasitemia | % Parasitemia |
|---|---|---|---|
| 0.1 | 5 | - | 83 |
| 0.3 | 5 | - | 79 |
| 1.0 | 5 | - | 77 |
| 3.0 | 5 | - | 82 |
| 10.0 | 5 | - | 85 |
| 0 | 10 | 26 | |

EXAMPLE 3

Infected mice were treated simultaneously with R-verapamil and chloroquine in the same way as in Example 1. Various doses of the two substances were used.

TABLE 3a

| R-verapamil + chlor. daily dose mg/kg | Number of mice | Mean control parasitemia | % parasitemia |
|---|---|---|---|
| 0.1 + 30.0 | 5 | - | 5 |
| 0.3 + 30.0 | 5 | - | 4 |
| 1.0 + 30.0 | 5 | - | 4 |
| 3.0 + 30.0 | 5 | - | 3 |
| 10.0 + 30.0 | 5 | - | 3 |
| 0 | 10 | 26 | |

TABLE 3b

| R-verapamil + chlor. daily dose mg/kg | Number of mice | Mean control parasitemia | % parasitemia |
|---|---|---|---|
| 0.1 + 60.0 | 5 | - | 3 |
| 0.3 + 60.0 | 5 | - | 2 |
| 1.0 + 60.0 | 5 | - | 2 |
| 3.0 + 60.0 | 5 | - | 0.7 |
| 10.0 + 60.0 | 5 | - | 0.4 |
| 0 | 10 | 26 | |

The parasitemia was almost completely suppressed with low doses of R-verapamil which were tolerated extremely well.

EXAMPLE 4

Test substance d) alone had only a very small effect on parasitemia

TABLE 4

| d) Daily dose mg/kg | Number of mice | Mean control parasitemia | % Parasitemia |
|---|---|---|---|
| 0.1 | 5 | - | 92 |
| 0.3 | 5 | - | 94 |
| 1.0 | 5 | - | 81 |
| 3.0 | 5 | - | 78 |
| 10.0 | 5 | - | 73 |
| 0 | 10 | | |

EXAMPLE 5

Combination of substance d) and chloroquine showed highly synergistic activity even in low doses in the test similar to that in Example 1.

TABLE 5

| d) + chlor. daily dose mg/kg | Number of mice | Mean control parasitemia | % Parasitemia |
|---|---|---|---|
| 0.1 + 0.3 | 5 | - | 47 |
| 0.3 + 0.3 | 5 | - | 37 |
| 1.0 + 0.3 | 5 | - | 36 |
| 3.0 + 0.3 | 5 | - | 33 |
| 10.0 + 0.3 | 5 | - | 14 |
| 0 | 10 | 23 | |

TABLE 6

| d) + chlor. daily dose mg/kg | Number of mice | Mean control parasitemia | % Parasitemia |
|---|---|---|---|
| 0.1 + 1.0 | 5 | - | 4 |
| 0.3 + 1.0 | 5 | - | 2 |
| 1.0 + 1.0 | 5 | - | 1 |
| 3.0 + 1.0 | 5 | - | 0.7 |
| 10.0 + 1.0 | 5 | - | 0.03 |
| 0 | 10 | 23 | |

The following table compares the therapeutically relevant properties of the substances according to the invention with R,S-verapamil, for example.

The AEI (activity enhancement index) is the ratio of the $ED_{99}$ for chloroquine alone and the $ED_{99}$ on addition of 10 mg/kg test substance. It is therefore a direct measure of the enhancement of the effect of the antimalarial on resistant pathogens when the substances according to the invention are administered simultaneously.

The table also includes three conventional tests for cardiovascular effects. In the first test on rat aortic strips the dose leading to 50% relaxation is determined and is a measure of the vasodilating, and thus blood pressure lowering, effect of a substance. The second test is of the lowering of blood pressure in the intact animal (rat) on intravenous administration.

The third test is of the effect of the test substance on the heart, likewise on the intact animal.

Indicated in each case is the dose of the test substance as a multiple of that of racemic verapamil which has the same cardiovascular effect.

The selectivity index in the table is the product of this factor and the AEI and thus indicates how much

weaker the cardiovascular effects of the test substance are than those of racemic verapamil when they are used in doses equally effective at breaking down resistance to antimalarials.

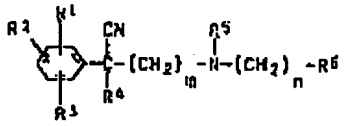
Compared with the dose of 240 to 480 mg of verapamil used in the therapy of hypertension, this table shows that the test substance dose which can be used for breaking down resistance has no cardiovascular activity.

TABLE 7

| Substance | ACI (10 µg/kg) | | Rat aortic strip | | Lowering of blood pressure | | ECG (AV block) rat | i.v. |
|---|---|---|---|---|---|---|---|---|
| | | | $ED_{50}$ | | $ED_{50}$ | | ED | |
| S,R-verapamil | 2.56 | - 1 | - 1 | S | - 1 | S | - 1 | S |
| R-verapamil | 4.15 | 1.6x | 6.3 | 10.2x | 3.5 | 8.9x | 4.6 | 7.6x |
| a) | 5.0 | 19.5x | 2.9 | 5.7x | 2.0 | 3.9x | 4.6 | 8.9x |
| b) | 4.5 | 1.8x | 7.7 | 13.5x | 2.7 | 4.9x | 2.15 | 3.9x |
| c) | 4.4 | 1.7x | 12.6 | 21.6x | 3.4 | 5.8x | 4.6 | 7.8x |
| d) | 4.20 | 1.6x | 6.0 | 9.8x | 4.3 | 6.9x | 2.15 | 3.4x |
| e) | 4.1 | 1.6x | 6.6 | 10.5x | 6.3 | 10x | 2.15 | 3.4x |
| f) | 3.44 | 1.3x | 5.7 | 7.7x | 4.3 | 5.6x | >2.15 | >2.8x |
| g) | 4.1 | 1.60x | 2.9 | 4.6x | 4.3 | 6.9x | 4.6 | 6.9x |
| h) | 3.7 | 1.44x | 11.0 | 15.8x | 2.0 | 2.80x | 2.15 | 3.0x |
| i) | 3.5 | 1.4x | - | - | 9.2 | 12.5x | - | - |
| k) | 3.3 | 1.3x | 6.3 | 8.1x | 20.0 | 26.0x | 2.15 | 2.8x |

We claim:

1. The use of those racemic and optically active substituted phenylacetonitriles of the formula I



I,

where

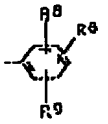
$R^1$, $R^2$ and $R^3$ are identical or different and are hydrogen, halogen, $C_{1-4}$-alkyl, $C_{1-4}$-alkoxy, trifluoromethyl, or two adjacent substituents $R^1$ and $R^2$ or $R^2$ are together $-CH_2-CH_2-CH_2-CH_2-$ or $-CH=CH-CH=CH-$,

$R^4$ is saturated or unsaturated alkyl or cycloalkyl of up to 15 carbon atoms or phenyl,

$R^5$ is hydrogen or $C_{1-4}$-alkyl,

m and n are each 2, 3 or 4, and

$R^6$ is saturated or unsaturated alkyl of up to 20 carbon atoms, $C_{4-8}$-cycloalkyl or



where $R^7$, $R^8$ and $R^9$ are identical or different and are hydrogen, halogen, $C_{1-4}$-alkyl, $C_{1-4}$-alkoxy, trifluoromethyl, or two adjacent substituents $R^7$ and $R^8$ or $R^8$ are together $-CH_2-CH_2-CH_2-CH_2$ or $-CH=CH-CH=CH-$, and the salts thereof with physiologically tolerated acids, which

a) have an $EC_{50}$ above $10^{-7}$ M the calcium antagonism test described in Advances in Myocardiology Vol. 4 (1983) on page 506,

b) have an $ED_{20}$ above 1.0 mg/kg in the test for lowering of blood pressure and

c) have a threshold dose above 2.0 mg/kg in the test for AV blockade,

for the preparation of drugs for breaking down resistance

to antimalarials.

2. A method for the treatment of malaria, which comprises treating the patient with an effective dose of a compound as claimed in claim 1 in combination with an antimalarial.

Abstract of the Disclosure: Substituted phenylacetonitriles are used for breaking down resistance to antimalarials is described.